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ATC ATC CGC AAC                                                    1212
Ile Ile Arg Asn
          404


(2) INFORMATION FOR SEQ ID NO: 3:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 39 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid (synthetic DNA)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CAACATGTCG TCAGTCATAT GTGTTTCCTG TGTGAAATT                          39
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What is claimed is:

1. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5–10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

2. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

3. The creatine amidinohydrolase of claim 2, which is obtained from *Escherchia coli* JM109 (pCRH273M2) (FERM BP-5375).

4. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

5. The creatine amidinohydrolase of claim 4, which is obtained from *Escherchia coli* JM109 (pCRH273M1) (FERM BP-5374).

6. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

7. The creatine amidinohydrolase of claim 6, which is obtained from *Escherchia coli* JM109 (pCRH273M3) (FERM BP-5376).

8. A method for producing the creatine amidinohydrolase of claim 1, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

9. The method of claim 8, wherein said microorganism is selected from the group consisting of *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374), *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375) and *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

10. A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 1, a sarcosine oxidase and a composition for the detection of hydrogen peroxide.

11. The reagent of claim 10, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore and a buffer.

12. The reagent of claim 11, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase and myeloperoxidase.

13. The reagent of claim 11, in which the chromophore comprises a hydrogen receptor and a coupler.

14. The reagent of claim 13, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

15. The reagent of claim 13, in which the coupler is an aniline derivative or a phenol derivative.

16. A method for determining creatine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim 10 with the sample.

17. A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 1, a sarcosine oxidase and a composition for the detection of hydrogen peroxide.

18. The reagent of claim 17, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore and a buffer.

19. The reagent of claim 18, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase and myeloperoxidase.

20. The reagent of claim 18, in which the chromophore comprises a hydrogen receptor and a coupler.

21. The reagent of claim 20, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

22. The reagent of claim 20, in which the coupler is an aniline derivative or a phenol derivative.

23. A method for determining creatinine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim 17 with the sample.]

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24. A method of preparing a creatine amidinohydrolase comprising:

(i) mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 to provide mutant nucleic acid sequences,

(ii) determining Km values of proteins encoded by the mutant nucleic acid sequences in a coupling assay using a sarcosine oxidase and a peroxidase,

(iii) selecting and isolating a desired mutant nucleic acid sequence that encodes a creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM,

(iv) expressing the desired mutant nucleic acid sequence in a host to produce creatine amidinohydrolase, and

(v) harvesting the produced creatine amidinohydrolase.

25. The method of claim 24, wherein the creatine amidinohydrolase has a molecular weight of about 43,000 (SDS-PAGE).

26. The method of claim 25, wherein the creatine amidinohydrolase has an isoelectic point of about 4.5.

27. The method of claim 26, wherein the creatine amidinohydrolase has an optimum temperature of about 40-50 °C (at pH of about 7.5).

28. The method of claim 27, wherein the creatine amidinohydrolase has an optimum pH of about 8.0-9.0 (at a temperature of about 37 °C).